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Dietary intervention with green dwarf banana flour (*Musa* sp AAA) prevents intestinal inflammation in a trinitrobenzenesulfonic acid model of rat colitis

Viviane Scarminio[a], Andrea C. Fruet[a], Aline Witaicenis[a], Vera L.M. Rall[b], Luiz C. Di Stasi[a,*]

[a]*Laboratory of Phytomedicines, Department of Pharmacology, Institute of Biosciences, Universidade Estadual Paulista, UNESP, Botucatu, SP, CEP 18618-970, Brazil*

[b]*Department of Microbiology and Immunology, Institute of Biosciences, Universidade Estadual Paulista, UNESP, Botucatu, SP, CEP 18618-970, Brazil*

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Abstract

Dietary products are among the therapeutic approaches used to modify intestinal microflora and to promote protective effects during the intestinal inflammatory process. Because the banana plant is rich in resistant starch, which is used by colonic microbiota for the anaerobic production of the short-chain fatty acids that serve as a major fuel source for colonocytes: first, green dwarf banana flour produces protective effects on the intestinal inflammation acting as a prebiotic and, second, combination of this dietary supplementation with prednisolone presents synergistic effects. For this, we used the trinitrobenzenesulphonic acid (TNBS) model of rat colitis. Our results revealed that the protective effect produced by a combination of 10% green dwarf banana flour with prednisolone was more pronounced than those promoted by a single administration of prednisolone or a diet containing 10% or 20% banana flour. This beneficial effect was associated with an improvement in the colonic oxidative status because the banana flour diet prevented the glutathione depletion and inhibited myeloperoxidase activity and lipid peroxidation. In addition, the intestinal anti-inflammatory activity was associated with an inhibition of alkaline phosphatase activity, a reduction in macroscopic and microscopic scores, and an extension of the lesions. In conclusion, the dietary use of the green dwarf banana flour constitutes an important dietary supplement and complementary medicine product to prevention and treatment of human inflammatory bowel disease.

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Keywords: Banana; Dietary products; Functional foods; Inflammatory bowel disease; *Musa* sp AAA; Ulcerative colitis; Trinitrobenzenesulfonic acid (TNBS); Rat

Abbreviations: ANOVA, analysis of variance; AP, alkaline phosphatase; GSH, glutathione; IBD, inflammatory bowel disease; IFN-γ, interferon-γ; MPO, myeloperoxidase; PPAR, peroxisome proliferator–activated receptors; RS, resistant starch; SCFA, short-chain fatty acids; TNBS, trinitrobenzenesulfonic acid.

1. Introduction

Inflammatory bowel disease (IBD) refers essentially to 2 different but closely related chronic intestinal disorders: Crohn disease and ulcerative colitis. Although much progress has been made in understanding the pathogenesis of human IBD, its etiology has not yet been defined. However, accumulating evidence suggests that this disease results from an inappropriate inflammatory response to intestinal microbes in a genetically susceptible host [1]. Furthermore, intestinal microbiota is linked to IBD pathogenesis because of its role in modulating

* Corresponding author. Department of Pharmacology, Institute of Biosciences, São Paulo State University, UNESP, Botucatu, São Paulo, SP 18618-970, Brazil. Tel.: +55 21 14 3811 6253; fax: +55 21 14 3815 3744.
E-mail address: ldistasi@ibb.unesp.br (L.C. Di Stasi).

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intestinal homeostasis and immunologic functions [2]. In fact, increasing experimental evidence supports the role of luminal bacteria in the initiation and development of the intestinal inflammatory process [3,4]. On the basis of these findings, 2 approaches have been used to modify intestinal microflora, the administration of probiotics or prebiotics, which are defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or the activity of limited bacteria in the colon [5].

Dietary fiber, defined as plant substances that resist hydrolysis by small bowel digestive enzymes, has been proven to be beneficial in maintaining remission in human ulcerative colitis, and this protective effect has been related to an increase in the luminal production of short-chain fatty acids (SCFAs), which are considered to be an important factor in the maintenance of healthy function in colorectal mucosa [6]. In fact, several studies have reported that some prebiotics including dietary fiber, germinated barley foodstuff, inulin, lactulose, and polydextrose exert beneficial effects in both human and experimental colitis models [7,8].

Banana is the fourth most important crop in developing countries, with a worldwide production of about 100 metric tons [9]. Fruits of the green dwarf banana (*Musa* sp AAA) are rich in starch granules containing 73.6% to 79.4% starch, and of the total amount of starch (14%), 47.3% to 54.2% is considered to be resistant starch [10-12]. Resistant starch is a nondigestible polysaccharide used as a dietary fiber that is resistant to digestion in the small intestine and used by colonic microbiota for the anaerobic fermentation production of SCFA [10-14].

Currently, the pharmacologic treatments for IBD include corticosteroids, aminosalicylates, immunomodulators, and anti–tumor necrosis factor-α antibodies, but these pharmacologic therapies result in serious adverse events, particularly after a long-term use. Because of these adverse effects and the chronic nature of IBD, there is dissatisfaction with current traditional therapies, which has led to an increase in the use of complementary and alternative medicine approaches including prebiotics and probiotics. The use of these compounds is currently estimated to be 49.5% [15,16].

Given that the green dwarf banana (*Musa* spp AAA) is an important source of resistant starch with several physiological effects consistent with those of dietary fibers and prednisolone, a drug that presents serious adverse effects from long-term use, two hypothesis of this study were evaluated. First: dietary supplementation with green dwarf banana flour produces protective effects on the intestinal inflammatory process acting as a prebiotic. Second: combination of dietary supplementation with prednisolone presents synergistic effects. For this purpose, we assayed the effects of the green dwarf banana flour and their combination with prednisolone in preventing the acute inflammatory response induced by trinitrobenzensulphonic acid (TNBS). In this experimental model, macroscopic, microscopic, and biochemical parameters were evaluated.

2. Methods and materials

2.1. Chemicals

All chemicals were supplied by Sigma (St Louis, Mo) and were freshly prepared for each animal administration or biochemical evaluation. The enriched diet with green dwarf banana flour was manufactured in the School of Medicine, São Paulo State University, UNESP, São Paulo, Brazil.

2.2. Plant material and diet preparation

Green dwarf banana fruits (*Musa* spp AAA) were collected in Botucatu City, São Paulo, Brazil, in December 2010. The plant was identified by taxonomists from Irina Felanova Gemtchjnicov Herbarium (Institute of Biosciences, São Paulo State University, UNESP), where a voucher specimen was deposited.

After collection, the green banana fruits were washed, chopped, and dried at 50°C for 72 hours in a hothouse with forced air circulation and renewal. After drying, the dried fruits were powdered to produce flour. For the preparation of the enriched diet, the flour was added at a ratio of 10% and 20% in previously sprayed Labina-Purine food for rodents. After homogenization, water was added to produce a paste. The paste was then placed in a pelletizer to produce diet pellets containing 10% or 20% green dwarf banana flour.

Table 1
Ingredient composition of the diets fed to rats (g/100 g)

Ingredients	Control diet	10% Banana diet	20% Banana diet
Protein mix	23.0	20.7	18.5
Mineral mix [a]	12.0	10.8	9.7
Fiber	5.0	4.5	3.6
Vitamin mix [b]	1.0	0.9	0.8
Fat	10.0	9.0	8.0
Fatty acids	5.5	4.95	4.4
Corn starch	32.0	28.8	25.7
Sugar mix	6.0	5.4	4.9
Soybean meal	2.5	2.25	2.0
Wheat bran	3.0	2.7	2.4
Banana flour [c]	–	10.0	20.0

[a] Mineral mixture provided the following amounts (in milligrams per kilogram): Mg, 1.7; Mn, 110.0; I, 1.0; Co, 2.0; Fe, 180.0; Zn, 110.0; Cu, 30.0, Se, 0.2, Na, 2.8, P, 8.5; and Ca, 13.0.

[b] Vitamin mixture provided the following amounts (in milligrams per kilogram per diet): vitamin A (25 600 UI); vitamin D_3 (4000 UI); vitamin E (82 mg); vitamin K (6.4 mg); vitamin B_{12} (40 μg); vitamin B_6 (11 mg); folic acid (13 mg); choline (2800 mg); biotin (0.16 mg); niacin (220 mg); thiamine (11 mg); and pantothenic acid (90 mg).

[c] Fruits of green dwarf banana (*Musa* sp AAA) containing starch (73.6%-79.4%), amylose (20.9%-23.5%), protein (2.61%-2.99%), soluble fiber (2.29%-2.49%), insoluble fiber (5.35%-5.39%), ash (3.44%-3.56), and traces of lipids [16].

The ingredient composition of the diets was calculated from the major nutrients of the normal Labina-Purine, taking into account the addition of 10% or 20% green dwarf banana flour (Table 1).

2.3. Animals

Male Wistar rats (weighing 180-200 g) from the Central Animal House, São Paulo State University–UNESP, Botucatu, São Paulo, Brazil, were housed in standard environmental conditions (21°C, 60%-70% humidity) under a 12-hour light/dark cycle and air filtration. The animals had free access to water and food (Purina-Labine, São Paulo, Brazil). All experimental protocols met the Guidelines of Animal Experimentation approved by the Commission of Ethics in Animal Experimentation (protocol number 042/04-CEAE), Institute of Biosciences, São Paulo State University–UNESP.

2.4. Experimental design

The rats were randomly assigned into 9 groups with 8 animals each. Two of the groups, a noncolitic group and a colitic group, received no treatment. Two additional noncolitic groups received an enriched diet with 10% and 20% dwarf banana flour for 21 days. Two colitic groups received an enriched diet with 10% and 20% dwarf banana flour for 14 days before colitis induction and 7 days thereafter. Two additional colitic groups received the enriched diet in the same conditions listed previously plus treatment with prednisolone administered at a dosage of 2 mg/kg for 3 days before colitis induction and 7 days thereafter. For comparison, the remaining group received only prednisolone (5 mg/kg) for 3 days before colitis induction and 7 days thereafter. Prednisolone was administered by means of an esophageal catheter (5 mL/kg). Rats from the noncolitic and nontreated colitic groups received water orally. Colitis was induced using the method originally described by Morris et al [17]. After fasting overnight, the animals were anesthetized with halothane. Under anesthesia, they were given 10 mg of trinitrobenzenesulfonic acid (TNBS) dissolved in 0.25 mL of 50% (vol/vol) ethanol by means of a Teflon (Dupont, Wilmington, Del) cannula inserted 8 cm into the anus. During and after TNBS administration, the rats were kept in a head-down position until they recovered from the anesthesia. Rats from the noncolitic (normal) group received 0.25 mL of saline. Animals from all groups were euthanized 7 days after colitis induction by an overdose of halothane.

2.5. Assessment of colonic damage

Animal body weights, the occurrence of diarrhea (as detected by perianal fur soiling), and total food intake for each group were recorded daily. Once the rats were killed, the colon was removed aseptically, placed on an ice-cold plate, and longitudinally opened. The luminal contents were then collected for the microbiological studies (see below). Afterward, the colonic segment was cleaned of fat and mesentery and blotted on a filter paper. Each specimen was weighed, and its length was measured under a constant load (2 g). The colon was scored for macroscopically visible damage on a 0 to 10 scale by 2 observers who were unaware of the treatment, in accordance with the criteria described by Bell et al [18].

Representative whole-gut specimens were taken from a region of the inflamed colon corresponding to the segment adjacent to the gross macroscopic damage and were fixed in 4% buffered formaldehyde. Cross sections were selected and embedded in paraffin. Equivalent colonic segments were also obtained from the noncolitic group. Full-thickness sections of 5 mm were obtained at different levels and were stained with hematoxylin and eosin. The histologic damage was evaluated by one observer who was blind to the experimental groups, according to the criteria described previously by Stucchi et al [19]. The colon was subsequently divided into different longitudinal pieces to be used for the following biochemical determinations: myeloperoxidase (MPO) activity, alkaline phosphatase (AP) activity, and total glutathione (GSH) content.

Myeloperoxidase activity was determined using the technique described by Krawisz et al [20]. The results are expressed as MPO units per gram of tissue. One unit of MPO activity was defined as the amount required to degrade 1 mmol of hydrogen peroxide per minute at 25°C.

Alkaline phosphatase activity was determined spectrophotometrically using disodium nitrophenylphosphate (5.5 mmol/L) as the substrate buffered in 50 mmol/L glycine with 0.5 mmol/L $MgCl_2$ at pH 10.5 [21]. The enzymatic activity is expressed as milliunits per milligram of protein [22].

Glutathione content was quantified with the recycling assay described by Anderson [23], and the results are expressed as nanomoles per gram of wet tissue.

2.6. Antioxidant activity

Additional in vitro experiments were performed to determine the antioxidant activity of different concentrations of green dwarf banana flour (1-100 μg/mL). The antioxidant activity was evaluated using an assay of lipid peroxidation in rat brain membranes [24] modified from the original protocol described by Gálvez et al [25]. The flavonoid quercetin was used as a reference and tested in the same assay system.

2.7. Microbiological studies

Luminal content samples were weighed, homogenized, and serially diluted in sterile 0.85% saline. Serial 10-fold dilutions of the homogenates were plated on Man, Rogosa, and Sharpeagar, a specific media for lactic acid bacteria, and were incubated under microaerobic conditions (5% CO_2) at 35°C for 120 hours. After incubation, the final colony count was reported as $\log_{10}$ colony-forming units per gram of fecal material.

Table 2
Effects of different treatments on damage score, extension of lesion, changes in colonic weight, incidence of adherence, and microscopic score in acute TNBS colitis

Experimental groups (n = 8)	Macroscopic score [a] (0-10)	Extension of lesion (cm) [b]	Colon weight (mg/cm)	Adherence [c] (%)	Microscopic score [a]
Noncolitic groups					
Nontreated	0 ***	0 **	99.39 ± 7.90 **	0 **	0 ***
10% diet	0 ***	0 **	102.07 ± 2.64 **	0 *	0 ***
20% diet	0 ***	0 **	112.12 ± 8.33 **	0 *	0 ***
Colitic groups					
TNBS control	6.0 (6-10)	2.85 ± 0.76	216.43 ± 32.67	50.0	14.0 (12-17)
10% diet	5.0 (0-6)	1.81 ± 0.76	160.37 ± 22.00	12.5 *	10.0 (9-11)
20% diet	2.0 (0-4) *	1.37 ± 0.35 *	138.30 ± 8.71 *	12.5 *	9.5 (8-13) *
10% diet + prednisolone	1.0 (0-5) *	1.16 ± 0.71 *	149.59 ± 13.47 *	12.5 *	8.0 (7-10) ***
20% diet + prednisolone	5.0 (3-6)	3.25 ± 1.01	171.00 ± 11.10	0.0 *	13.0 (11-13)
Prednisolone	3.0 (0-4) *	1.41 ± 0.29 *	162.21 ± 8.14	37.5^{+}	11.0 (8-14) *

[a] Score data are expressed as the median (range) and analyzed by Kruskal-Wallis test.
[b] Extension of lesion and colonic weight data are expressed as the means ± SEM and analyzed by ANOVA followed by post hoc test of Dunnett.
[c] Adherence is expressed in percentage and analyzed by χ^2 test.
* $P < .05$ vs TNBS control group.
** $P < .01$ vs TNBS control group.
*** $P < .001$ vs TNBS control group.

2.8. Statistical analyses

The results are expressed as means ± SEM values, and differences between the means were tested for statistical significance using a 1-way analysis of variance (ANOVA) and post hoc least significance tests. Nonparametric data (scores) are expressed as the median (range) and were analyzed with the Kruskal-Wallis test. Differences between proportions were analyzed with the χ^2 test. Statistical significance was set at $P < .05$.

3. Results

Trinitrobenzenesulfonic acid administration resulted in colonic inflammation, which was demonstrated after 7 days by severe necrosis of the mucosa, typically extending 2.8 to 4.9 cm along the colon, bowel wall thickening, and hyperemia (Table 2). This inflammatory process was associated with an increase in the colonic weight/length ratio, incidence of the adherence of the colon to adjacent organs (Table 2), and signs of diarrhea in 100% of the colitic. A histologic assessment of colonic samples from the TNBS control group revealed severe transmural disruption of the normal architecture of the colon, extensive ulceration, and inflammation involving all the intestinal layers of the colon, giving a score value of 14.0 (Table 2). Biochemically, the colonic damage was characterized by a reduction in colonic GSH levels and an increase in MPO and AP (Table 3) activities when compared with noncolitic animals.

The treatment of noncolitic animals (healthy rats) with the diet enriched with 10% and 20% dwarf banana flour for 21 days showed no effects on the clinical, macroscopic, and microscopic parameters analyzed. The measurements taken from these animals were similar to those taken from the noncolitic group that received only vehicle (Table 2). No statistical differences were observed in food consumption or weight gain between the experimental groups that received the enriched diet and those that received the normal diet.

Feeding a 20% dwarf banana diet to colitic rats resulted in a lower colonic damage score, with a significant reduction in the extension of lesion, the colonic weight/length ratio, and the incidence of adherence to adjacent organs compared with the TNBS control group (Table 2). The histologic studies confirmed this intestinal anti-inflammatory effect with a lower microscopic damage score of 9.5 (Table 2) and a pronounced recovery in the colon cytoarchitecture with a

Table 3
Effects of banana diet supplementation (10% and 20%) and their combined effects with prednisolone (2 mg/kg) on GSH content, MPO, and AP in TNBS colitis model

Experimental groups (n)	GSH	MPO	AP
Noncolitic groups			
Nontreated (8)	2302 ± 86.13 **	243.66 ± 14.58 **	4.04 ± 0.64 **
10% diet (8)	2373 ± 109.15 **	304.94 ± 14.38 *	2.27 ± 0.20 **
20% diet (8)	2278 ± 117.27 **	223.29 ± 11.69 *	5.97 ± 0.46 *
Colitic groups			
TNBS control (8)	1679 ± 90.87	754.62 ± 115.70	11.37 ± 2.07
10% diet (8)	2070 ± 121.81 *	778.27 ± 158.34	9.67 ± 0.88
20% diet (8)	2621 ± 133.89 **	299.43 ± 22.40 *	3.12 ± 0.37 *
10% diet + prednisolone (8)	2257 ± 72.11 **	388.87 ± 37.21 *	3.13 ± 0.33 **
20% diet + prednisolone (8)	2134 ± 113.84 *	622.79 ± 177.32	9.14 ± 0.81
Prednisolone (8)	2210 ± 46.45 **	576.07 ± 71.16	6.77 ± 1.00 *

Data are expressed as means ± SEM. Differences were assessed using ANOVA, followed by post hoc tests of Dunnett.
* $P < .05$ vs TNBS control group.
** $P < .01$ vs TNBS control group.

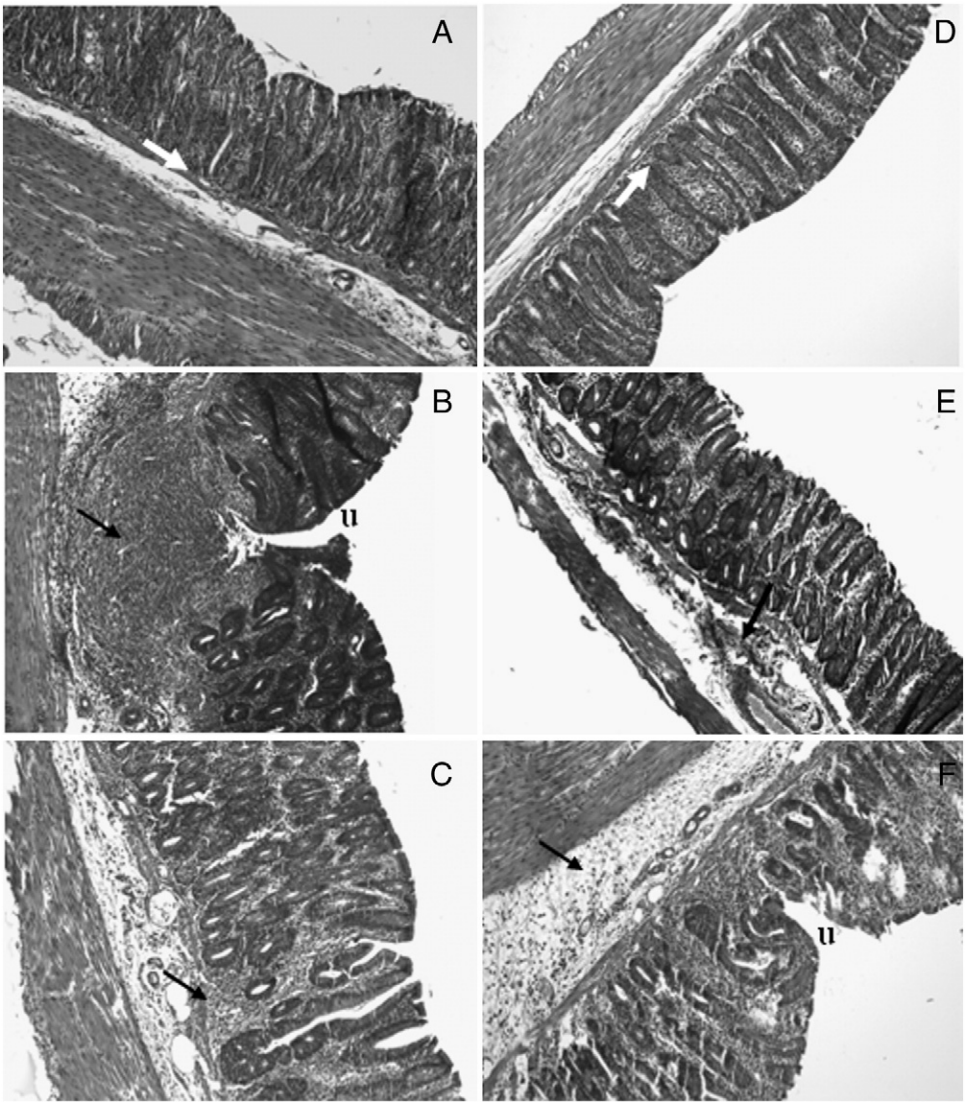

Fig. 1. Photomicrography of colon tissue from different groups: noncolitic (A), TNBS control (B), prednisolone (2 mg/kg) (C), 10% banana flour diet (D), 20% banana flour diet (E), and 10% banana diet + prednisolone (2 mg/kg) (F). The noncolitic group (A) had normal colon histology, including numerous tubular glands with goblets cells (arrow). The lumen epithelium was intact with typical morphology. B, TNBS control group showing ulceration (u). The goblets cells were not frequently in the wall of the gland. Severe inflammation in the mucosa and submucosa was observed, with many lymphocytes in the lamina propria (arrow). In the prednisolone-treated group (C), we observed a mild ulceration in the mucosa (u) and a reduced number of inflammatory cells (arrow) compared with the TNBS control group. In the group receiving the 10% banana flour diet (D), no inflammatory cells were detected in the mucosa or submucosa, and normal colon histology was observed, including tubular glands with goblets cells (arrow). In the group treated with the 20% banana flour diet (E), the presence of inflammatory cells in the submucosa was still evident (arrow), but the area was small compared with the TNBS control group. In animals treated with a 10% banana diet + prednisolone (F), we observed a mild ulceration in the mucosa (u) and inflammatory cells in the submucosa (arrow), but this number was, thus, small compared with the TNBS control group.

reduction of the leukocyte infiltration compared with the TNBS control group (Fig. 1). The intestinal anti-inflammatory effect was also demonstrated biochemically by the maintenance of the colonic GSH level (Table 3). The observed decrease in leukocyte infiltration in our histologic studies was also demonstrated by the reduction in the MPO activity (Table 3). Indeed, AP activity was also significantly reduced in rats treated with a diet enriched with 20% dwarf banana flour, in contrast with the increase of AP activity that occurred in the TNBS control group (Table 3).

Colitic rats that received the 10% dwarf banana flour diet showed moderate protective effects on the incidence of colon adherence and GSH colon content only (Table 3). No significant effects were observed in the damage score, the microscopic damage score, the extent of colonic lesions, the colonic weight/length ratio, or the MPO and AP activities (Tables 2 and 3).

When colitic rats were treated with a combination of the enriched diet and prednisolone, protective effects were observed using 10% dwarf banana flour. The combined

treatment using the diet containing 20% dwarf banana flour showed significant effects only in the reduction of the incidence of colon adherence to adjacent organs and counteracting the GSH depletion induced by the colonic inflammatory process (Tables 2 and 3). The combined treatment using the 10% dwarf banana flour diet and prednisolone provided a beneficial effect in colitic rats, as demonstrated by the greater reduction in the macroscopic damage score values associated with a reduction in the extent of lesions, the colonic weight/length ratio, adherence of the colon to adjacent organs, and the microscopic damage score (Table 2). This protective effect was confirmed by histologic studies that showed a pronounced recovery of colon cytoarchitecture accompanied by mild ulceration in mucosa and a reduction of the inflammatory cells in the submucosa (Fig. 1). The reduced level of inflammatory cell migration was also confirmed by a reduction in the MPO activity (Table 3). In addition, this drug combination was able to counteract GSH depletion and reduce colon AP activity (Table 3).

The reference drug used, prednisolone, showed anti-inflammatory effects, as demonstrated by the reduction in the macroscopic and microscopic damage scores and the extent of lesions (Table 2). This protective effect was also biochemically related to the maintenance of the GSH content and a reduction of AP activity (Table 3). Prednisolone showed no effects on the MPO activity, the occurrence of adhesions between the colon and adjacent organs, or the colonic weight/length ratio (Tables 2 and 3).

No statistical difference in the lactic acid bacteria count was found between normal and colitic rats. The enriched diet with 10% and 20% green dwarf banana flour did not alter lactic acid bacteria counts in noncolitic or colitic animals (data not shown).

The in vitro experiments performed show that green dwarf banana flour exerts a concentration-dependent inhibitory effect on the lipid peroxidation induced in rat brain membranes, with an IC_{50} (50% inhibitory concentration) value of 67.61 ± 2.35 μg/mL. The corresponding IC_{50} value of quercetin was 0.41 ± 0.17 μg/mL.

4. Discussion

Current pharmacologic treatment of IBD includes anti-inflammatory drugs (aminosalicylates and corticosteroids), immunosuppressants, biological agents, antibiotics, and drugs for symptomatic relief [26], but these pharmacologic therapies result in unwanted adverse effects, particularly after long-term use. Glucocorticoids, particularly prednisolone, are not a viable long-term solution for IBD management because they produce adverse effects and damage body parts and their function from long-term use [27]. Thus, a combination of products that improve the anti-inflammatory activity of glucocorticoids would be an important approach for IBD treatment. The present study was designed to evaluate novel experimental interventions using green dwarf banana flour as a potential dietary product because this plant is rich in resistant starch, a type of starch that may be applied to the prevention of intestinal inflammatory diseases [28].

In the first set of experiments, we evaluated the effects of an enriched diet containing 10% and 20% dwarf banana flour, and our data demonstrated that the diet containing 20% banana flour prevented the intestinal inflammatory process induced by TNBS; whereas the diet containing 10% banana flour only partially prevented this inflammatory process. The preventive effect promoted by the 20% banana flour diet was demonstrated by the significant reduction in the macroscopic parameters evaluated and confirmed by histologic analysis, the counteraction of GSH content, and the reduction of the MPO and AP activities. Glutathione is an important endogenous antioxidant peptide that is reduced in the course of the intestinal inflammatory process, and MPO is considered to be a marker of neutrophil infiltration. The effects on these biochemical mediators are indicative of antioxidant and anti-inflammatory activity, respectively. Although the anti-inflammatory activity of prednisolone was observed, the prevention of the inflammatory process after supplementation with the 20% banana flour diet was more pronounced in all parameters analyzed, particularly for the damage score (2.0 vs 3.0), the incidence of adherence (12.5% vs 37.5%), the microscopic damage score (9.5 vs 11.0), GSH content (2621 ± 133.89 vs 2210 ± 46.45), and AP activity (3.12 ± 0.37 vs 6.77 ± 1.00). The colonic weight/length ratio and MPO activity were significantly reduced after treatment with the 20% banana flour diet, but these effects were not significantly altered after the administration of prednisolone.

In the second set of experiments, we evaluated the effect of banana flour supplementation on the intestinal anti-inflammatory activity of prednisolone to determine whether banana flour improves the pharmacologic activity of this glucocorticoid that is currently used in treatment of human IBD. Our results revealed that the combined use of a 10% banana flour diet with prednisolone was effective for preventing the intestinal inflammatory process, as demons trated by the improvement in the macroscopic, microscopic, histologic, and biochemical inflammatory parameters evalu ated. This preventive effect was more pronounced than those observed after a single administration of prednisolone, the use of the 10% and 20% banana flour diet alone, or the 20% banana flour diet combined with prednisolone.

The fruits of the green dwarf banana are rich in starch, primarily presented as resistant starch [10], which can act as a substrate yielding high levels of butyrate [28], an SCFA that improves gastrointestinal health, immune surveillance, and the growth and differentiation of enterocytes [6,29,30]. Recent studies have shown that after 7 days of supplementation with resistant starch, chronically inflamed rats had the same butyrate uptake as rats fed on the basal diet [30]. In fact, prebiotic foodstuffs derived from resistant starch were

suggested to be effective in the amelioration of colitis in both clinical and animal studies [28,30]. Green dwarf banana flour has been chosen as a starch source because of the high content of resistant starch, whereas banana fruit is considered to be one of the few sources of this resistant starch available in an ordinary meal [11,31]. In addition to the great value of resistant starch as a source of butyrate, resistant starch 2, a starch type present in green dwarf bananas, is also rich in amylose, which increases SCFA production and *Bifidobacterium* spp and *Lactobacillus* spp growth in the gut [32-35]. In our experimental conditions, the intestinal anti-inflammatory effect of the banana flour diet was not related to prebiotic properties because no improvement in bacterial growth and development was observed. However, the methods used to determine bacterial growth and development are limited, and new studies are necessary, particularly using other experimental models of colitis, such as dextran sulfate sodium, and a more appropriate and specific culture medium.

The role of the reactive metabolites of oxygen and nitrogen in the pathophysiology of IBD has been reported [36]. Although the specific pathways leading to cellular damage are not completely understood, oxidative stress is a potential etiologic and/or triggering factor for IBD, and antioxidant therapy can constitute an interesting approach in the regulation of this intestinal inflammation condition [37]. In fact, the anti-inflammatory effect of 5-aminosalicylic derivatives has been partially attributed to their free radical scavenger and antioxidant properties [36]. In addition, some herbal therapies have been demonstrated to have the ability to ameliorate IBD via their antioxidant capacity, reducing indicators of lipid peroxidation, such as MPO, malondialdehyde, and thiobarbituric acid reactive substances, or improving antioxidant power by increasing GSH, catalase, and superoxide dismutase [38]. Our study shows that green dwarf banana flour shows antioxidant activity in vitro, demonstrated by the inhibition of lipid peroxidation in rat brain membranes, and in vivo, demonstrated by counteracting colonic GSH depletion. The observed effect exerted by the diet enriched with banana flour in preserving the colonic mucosa from oxidative insult may be a factor in diminishing the neutrophil infiltration that occurs in response to TNBS. Brazilian dwarf banana fruit has been described as a rich source of several potent and common antioxidant compounds such as vitamin C, α-carotene, β-carotene, and lutein [39]. Other studies have reported the antioxidant activity of bananas (*Musa* sp AAA), demonstrated by a decrease in lipid peroxides and an increase in GSH content in the rat liver [40]. Flavonoids from *Musa paradisiaca* produce antiperoxidative activity, as demonstrated by the reduction of malondialdehyde and hydroperoxides concentrations and an increase of the catalase and SOD activities in the rat liver, kidney, and heart [41,42].

On the basis of our results, we can conclude that diet supplementation with 20% green dwarf banana flour and the combination use of a 10% banana flour diet with prednisolone prevents TNBS-induced colonic damage in rats. This effect may be associated with an improvement in intestinal oxidative stress probably because of the antioxidant properties of bananas. In addition, the beneficial properties of the green dwarf banana flour may also be attributed to the described presence of potent antioxidant compounds, such as vitamin A, carotenes, and lutein, and fermentation products, such as resistant starch and amylose, in this plant. Indeed, the protective effect was not related to prebiotic properties, given that the green dwarf banana flour did not produce changes in total content of lactic bacteria. Indeed, although the combination of the 10% green dwarf banana flour diet with prednisolone produced better effects than other tested products, this effect was not synergistic because no statistical differences among the treated groups were found. In conclusion, the use of green dwarf banana flour constituted an important dietary supplement and complementary medicine product in the prevention and treatment of human IBD. However, because of the limitations of this study, further research is necessary to better understand the intestinal anti-inflammatory properties of this dietary intervention and its combination with glucocorticoids using other methods of colitis induction and the evaluation of additional inflammatory mediators.

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